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GENETIC DISORDERS - DEVELOPMENT

A novel frameshift mutation induced by an adenosine insertion in the polycystic kidney disease 2 (*PKD2*) gene

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A novel frameshift mutation induced by an adenosine insertion in the polycystic kidney disease 2 (*PKD2*) gene. Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common Mendelian disorders and is genetically heterogeneous. Linkage studies have shown that the majority (~85%) of ADPKD cases are due to mutations in *PKD1* on chromosome 16p13.3, while mutations in *PKD2* on chromosome 4q21-q23 are thought to account for most of the remaining cases. In this report, we describe the mutation in a large four-generation ADPKD family (TORPKD77) which we had mapped to the *PKD2* locus by linkage analysis. In this family, we screened for mutations by directly sequencing two nested RT-PCR fragments (PKD2N1 and PKD2N2) that cover ~90% of the *PKD2* open reading frame. In the affected members, we identified a novel single adenosine insertion (2160InsA) in the PKD2N2 fragment. This mutation occurred in the polyadenosine tract (nt2152-2159) of exon 11 and is predicted to result in a frameshift with premature translation termination of the *PKD2* product, polycystin 2, immediately after codon 723. The truncated polycystin 2 is predicted to lack the calcium-binding EF-hand domain and two cytoplasmic domains required for the homodimerization of polycystin 2 with itself and for the heterodimerization of polycystin 2 with polycystin 1.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common Mendelian disorders and affects approximately 1 in 1,000 live births [1, 2]. Clinically, it is characterized by progressive formation and enlargement of cysts, typically leading to end-stage renal disease (ESRD) in late middle age. Overall, ADPKD accounts for about 5 to 10% of ESRD [1, 2]. The findings of extra-renal cysts together with increased incidence of mitral valve prolapse, inguinal hernia, colonic diverticulae, and intracranial arterial aneurysms suggest that ADPKD is a systemic disease of connective tissue [1].

ADPKD is genetically heterogeneous [1–6]. Linkage studies have shown that the majority (~85%) of ADPKD cases are due to mutations in *PKD1* on chromosome 16p13.3, while mutations in *PKD2* on chromosome 4q21-q23 are thought to account for most of the remaining cases [3, 4]. Recently, a small number of families have been reported to be unlinked to both the *PKD1* and *PKD2* loci, suggesting the existence of at least one more locus (*PKD3*) [5, 6]. Locus heterogeneity is now known to contribute to the differences in disease severity in ADPKD, with *PKD1*-linked families having more severe disease. The mean age of onset of ESRD among affected members from *PKD1*-linked families is 56 years. In contrast, the mean age of onset of ESRD among affected members from *PKD2*-linked families is 70 years [3, 4].

Both *PKD1* and *PKD2* have been cloned recently [7–9]. For *PKD1,* mutation screening has been difficult due to its size (that is, ~13 kb of open reading frame) and complexity (~75% of the gene is duplicated) [7, 8]. So far, ~25 different mutations in the unique and part of the duplicated regions of *PKD1* have been reported from the 15 to 20% of patients being screened [10–16]. In comparison, *PKD2* is a single copy gene with an open reading frame of ~3 kb [9]. However, *PKD2* mutations are less common, and only four have been described to date [9, 17]. Here, we report a novel mutation resulting from a single adenosine insertion in the polyadenosine tract of exon 11 in *PKD2*.

METHODS

Clinical ascertainment and sample collection

Seventeen members (i.4. III:3, III:11-14, IV:1-11, IV:13) of the TORPKD77 family were recruited in the present study (Fig. 1). For those members known to be affected with ADPKD, their diagnosis was confirmed by reviewing their clinical records. All other members (including spouses) whose affection status was unknown underwent a screening ultrasound. In addition, information with respect to the deceased members of the family (such as age at death, affectedness status, and requirement of ESRD treatment) was obtained by interviewing several knowledgeable family members. Blood samples were obtained from all participating members and genomic DNA was extracted from peripheral blood lymphocytes by the salting-out method [18]. Total RNA was extracted from peripheral blood lymphocytes of an affected (IV:10) and an unaffected member (III:12) [19]. The research protocol used in the present study was reviewed and approved by the Human Subject Review Committees at the University of Toronto.

Key words: Mendelian disorder, family genetics, cyst enlargement, end-stage renal disease, mutation in *PKD2*.

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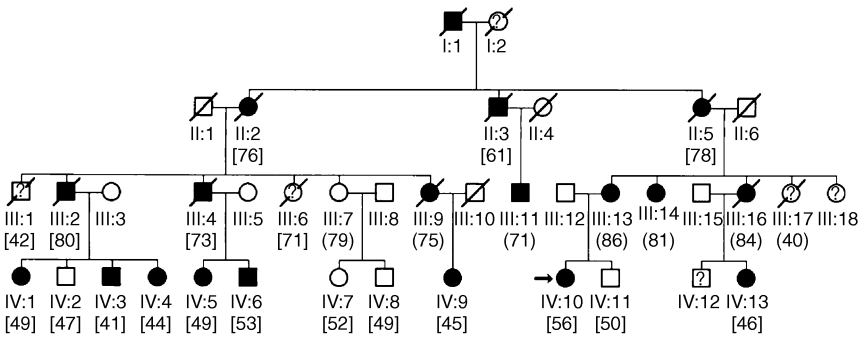

Fig. 1. Pedigree structure of TOR-PKD77. Circles represent females, and squares represent males. All affected individuals are indicated by blackened symbols, all unaffected individuals are shown in open symbols, and all individuals whose affection status is unknown are shown with (?). A diagonal line through the symbol indicates the individual is deceased. The age of death is shown in [ ], and the age at the last ultrasound is shown in ( ). The proband is indicated by the arrowhead.

Table 1. Results of pairwise linkage analysis of ADPKD with markers at PKD1 and PKD2 loci

	Lod score at recombination fraction (θ) of:							
	0.00	0.01	0.05	0.10	0.20	0.30	0.40	0.50
PKD1 markers:								
ADPKD vs.								
KG8	−1.60	−0.92	−0.36	−0.15	−0.02	0.00	0.00	0.00
142P	−3.22	−2.17	−0.98	−0.51	−0.11	−0.02	0.04	0.00
D16S291	−5.60	−4.74	−2.80	−1.69	−0.72	−0.29	−0.09	0.00
PKD2 markers:								
ADPKD vs.								
D4S1563	0.21	0.20	0.18	0.15	0.090	0.04	0.02	0.00
SPP1	4.55	4.47	4.12	3.67	2.72	1.71	0.72	0.00

Genotyping and linkage analysis

Genotyping of PKD1 (that is, KG8, I42P, and D16S291) and PKD2 (D4S1563, and SPP1) markers was performed using published methods [9, 13, 20, 21]. All markers are dinucleotide repeat markers except for I42P, which is a VNTR (variable number of tandem repeat) marker [13]. All dinucleotide repeat markers were genotyped by ^{32}P α-dCTP labeling of the PCR products and analyzed after separation by polyacrylamide gel electrophoresis [9, 20, 21].

Two-point "maximum likelihood" linkage analysis was performed using the FASTLINK suite of programs [22]. Multipoint analysis was performed using LINKMAP. We used a dominant model with a disease gene frequency for PKD1 and PKD2 of 0.001 and 0.0002, respectively [5, 21]. Allele frequencies were calculated from the alleles observed in married-in individuals from the pedigree using PEDMANAGER, a computer package developed at the Whitehead Institute (Reeve-Daly MP, unpublished), and sex average recombination fractions were used. Lod scores were calculated at the known recombination fractions between markers and either PKD1 or PKD2 locus. Age-dependent penetrance was incorporated using four liability classes assigned to unaffected individuals, with penetrances of 0.5, 0.7, 0.95, and 0.99 for members over age 20, 30, 50, and 55 years, respectively [23].

PKD2 mutation screening

To screen for *PKD2* mutation, two nested RT-PCR fragments (PKD2N1 and PKD2N2), which covered 90% of the *PKD2* coding sequence (nt 367-3020), were amplified from the cDNA of an affected and an unaffected individual, and then sequenced directly. Reverse transcription for first strand cDNA synthesis was performed in a 20 μl reaction with 2.5 μg of total RNA, oligo (dT) primers, and Superscript II (GibcoBRL) at 42°C, according to the manufacturer's instructions. Reverse transcription-polymerase chain reaction (RT-PCR) was performed in a 50 μl reaction using the Expand™ Long Template PCR system (Boehringer Mannheim, Mannheim, Germany). The primers for the first round template (PKD2X) and the nested fragments (PKD2N1 and PKD2N2), and their annealing temperatures are shown in Table 2. Buffer 1 with the addition of 5% DMSO was used and all PCR was performed with "hot-start." Direct sequencing of the PCR products were performed on both strands using the fluorescent dideoxy terminator method and analyzed using an ABI 373 DNA sequencer (Applied Biosystems). The primers used for sequencing are shown in Table 2.

Allele specific oligonucleotide hybridization

Segregation analysis of the mutation with ADPKD was performed by amplifying the genomic region containing the mutation, and then the PCR products were analyzed by allele specific oligonucleotide (ASO) hybridization. The primers used for the genomic PCR were: IF10: 5′-AAACCAAGTCTTTTATTTTT-TCTC-3′ and IR11: 5′-GGGCTAGAAATACTCTTATCACC-3′. The PCR products were first denatured and then blotted in duplicate on to two Hybond-N+ membranes. Two oligonucleotide probes [that is, wild-type (W): 5′-ACTGAAAAAAAATAC-CGTG-3′ and mutant (M): 5′-CTGAAAAAAAAATACCGTG-3′] encompassing the site of mutation were end-labeled with ^{32}P-γ ATP by T4 polynucleotide kinase. Each probe was separately hybridized to one membrane in 5 × SSC/5× Denhardt's solution/0.5% SDS at 45°C for one hour. Each membrane was then washed with 2 × SSC/0.1% SDS for 10 minutes each at room temperature, at 52°C, and at 54°C, and exposed for autoradiography.

RESULTS

Figure 1 shows the TOR-PKD77 pedigree. The proband (IV:10) was a 56-year-old female found to have ADPKD by ultrasound screening, but was otherwise asymptomatic. Three affected members (III:2, III:9, III:11) from her family had developed ESRD and five other affected members (II:2, II:3, II:5, III:4, III:16) died with less severe disease without requiring dialysis. The mean age at ESRD or death in these eight affected individuals was 74 years (95% confidence interval, 69 to 80 years). Two other affected members (III:13 and III:14) have moderate renal insufficiency with creatinine clearances in the 40 to 60 ml/min range at age 81 and 86 years. None of the affected members from the fourth generation has impaired renal function. Most of the

Table 2. Oligonucleotide primers used for RT-PCR and sequencing

Name	Sequence (5′-3′)	Annealing temperature °C	Position of PCR product *nt*	PCR product size *kb*
RT-PCR primers				
PKD2X(F)	TCCCCTTCTCCTCCGCTCTC	60	[286-3033]	~2.75
PKD2X(R)	CAGCAATTCAGGACAGCCACTTC			
PKD2N1(F)	GAGGAGGTGGAAGGGGAAGAA	64	[367-1621]	~1.25
PKD2N1(R)	CTGACAGCACAACGATCACAACA			
PKD2N2(F)	GTGGTCAGGTTATTGGTTGAA	56	[1378-3020]	~1.64
PKD2N2(R)	CAGCCACTTCCTCACTTATTAGA			
			Position of sequencing Primer *(nt)*	
Sequencing primers				
PKD2N1(F)	GAGGAGGTGGAAGGGGAAGAA		[368-397]	
PKD2S1(F)	CGGATGATGTCACAGCTCTTCCTA		[817-840]	
PKD2S2(R)	CATCCAATAAGGAGCCTTCTGTGA		[911-934]	
PKD2S3(F)	GTGGTCAGGTTATTGGTTGAA		[1378-1398]	
PKD2S4(R)	CTGACAGCACAACGATCACAACA		[1599-1621]	
PKD2S5(F)	CTGACTTGGCACAGCAGAA		[2132-2150]	
PKD2S6(R)	GCCTCAATCTCTGCATCAGTA		[2316-2336]	
PKD2S7(F)	GAGCATTCCATCGGCAGCATA		[2614-2634]	
PKD2N2(R)	CAGCCACTTCCTCACTTATTAGA		[2998-3020]	

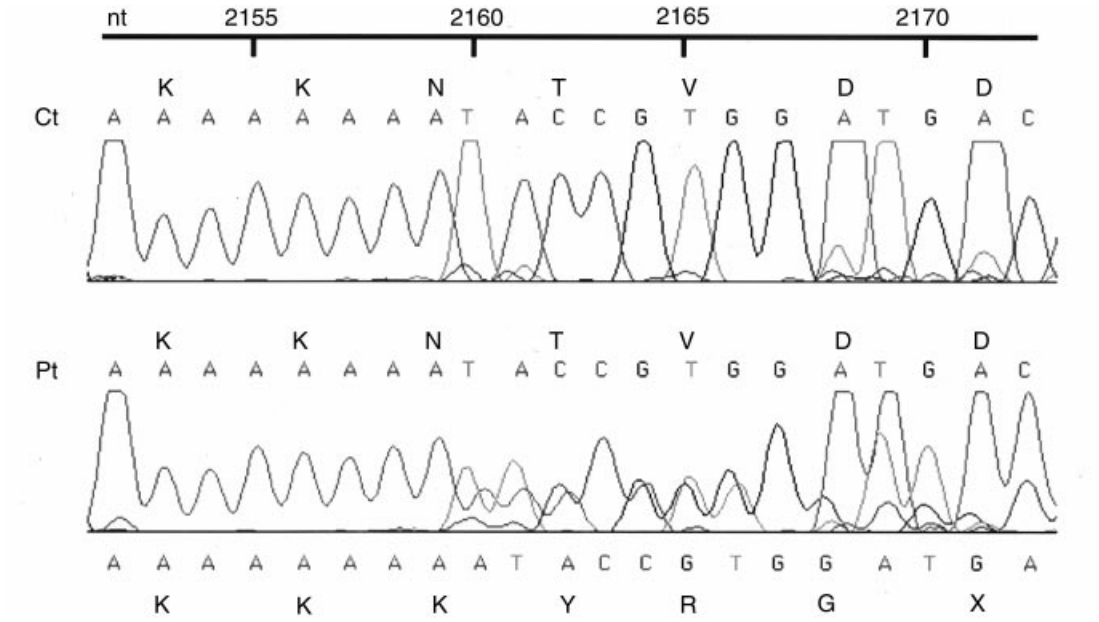

Fig. 2. Sequence tracings of genomic PCR products showing the TOR-PKD77 *PKD2* mutation. The sequence of the normal allele is shown above the electropherograms of the control (Ct; III:12) and patient (Pt; IV:10) samples, while the mutant sequence is shown below the patient tracing. The patient sample reveals a heterozygous mutation for a single adenosine insertion at nucleotide 2160 immediately after the polyadenosine tract (nt 2152-2159) on exon 11. This mutation is predicted to result in a frameshift with premature translation termination of polycystin 2, immediately after codon 723 (amino acid codes are: K, lysine; N, asparagine; T, threonine; V, valine; D, aspartic acid; Y, tyrosine; R, arginine; G, glutamine; X, stop codon).

affected members also had liver cysts on ultrasound. There was no history of ruptured intracranial arterial aneurysm in this family.

We genotyped three polymorphic markers at the *PKD1* locus. Both KG8 and I42P are intragenic markers of *PKD1* [13, 20], while D16S291 is a marker within ~0.2 cM from *PKD1* [20]. All three markers gave negative lod scores (Table 1). Multi-point linkage analysis using these three markers yielded a lod score of −4.53 with an exclusionary interval of 5 cM, thus excluding linkage of this pedigree to *PKD1*. We also genotyped two other markers, D4S1563 and SPP1, which are within 1 cM telomeric and centromeric from *PKD2*, respectively [9, 21]. While D4S1563 was not very informative in this family, SPP1 provided strong evidence supporting linkage of the disease locus in this family to *PKD2* ($Z_{max} = 4.55$, $\theta = 0.00$; Table 1). In addition to the linkage results, the mild disease observed in the older affected members further supported that this family was *PKD2*-linked [3, 4]. We therefore proceeded to screen for mutations in *PKD2*.

Two nested RT-PCR fragments [PKD2N1 (nt 367-1621) and

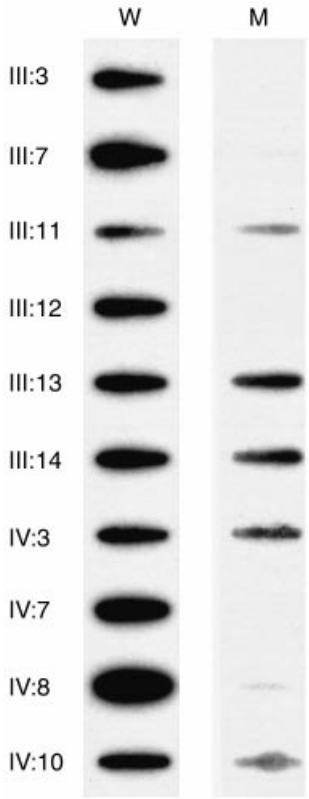

Fig. 3. Representative results of segregation analysis by allele specific oligonucleotide (ASO) hybridization. Polymerase chain reaction (PCR) products amplified from the genomic region containing the *PKD2* mutation were denatured and blotted in duplicate on to two Hybond-N+ membranes. ASO hybridization was performed with two oligonucleotide probes [wild-type (W), 5′-ACTGAAAAAAAATACCGTG-3′; and mutant (M), 5′-CTGAAAAAAAAATACCGTG-3′], which detect the normal and mutant sequence, respectively. The wild-type probe hybridized to the PCR products from both the affected and unaffected individuals, while the mutant probe hybridized only to the PCR products from the affected patients (that is, III:11, III:13, III:14, and IV:3,10).

PKD2N2 (nt 1378-3032)], which contained 90% of the open reading frame of *PKD2*, were amplified from the cDNA of the proband (IV:10) and an unaffected member (III:12). Both RT-PCR products were sequenced directly. No mutation was detected in the PKD2N1 fragment. In contrast, electropherograms of the PKD2N2 fragment from the proband revealed a single adenosine insertion at nucleotide 2160 immediately after the polyadenosine tract (nt 2152-2159) on exon 11 of *PKD2* [20]. This mutation was confirmed by re-sequencing exon 11 from RT-PCR products on the non-coding strand and from the genomic template. This single base insertional mutation is predicted to result in a frameshift with premature translation termination of polycystin 2, immediately after codon 723 (Fig. 2). To further assess the segregation of this mutation with ADPKD in the TOR-PKD77 pedigree, we amplified exon 11 from genomic DNA in all study subjects and from 40 normal Caucasian controls, and analyzed the PCR products by allele-specific oligonucleotide hybridization. These data confirmed that all the affected subjects, but none of the spouses and at-risk escapees of age $\geq$ 50 years, were heterozygous for the 2160InsA mutation (Fig. 3). The 2160InsA mutation was absent from all 80 normal chromosomes.

DISCUSSION

In the present study, we detected a novel frameshift mutation due to a single adenosine insertion (2160InsA) in the polyadenosine tract (nt 2152-2159) of exon 11 of *PKD2* [24]. Simple sequence repeats, consisting of repeating units of one to five base pairs, are abundant and highly dispersed in the eukaryotic genomes [25, 26]. Many of these simple sequence tracts are also highly polymorphic and recent studies suggest that they are prone to mutations by "slipped strand mispairing" [25–27]. This may occur when the normal pairing between the two complementary strands is altered by staggering of the repeats on the two strands, leading to incorrect alignment of the repeats. Upon DNA replication, small insertions or deletions may result that can then be passed on to the germline [26, 27]. Thus, "slipped strand mispairing" of the polyadenosine tract in exon 11 of *PKD2* may provide a plausible mechanistic explanation for the observed mutation.

The predicted *PKD1* product, polycystin 1, is a large glycoprotein that contains several extracellular motifs indicative of a role in cell-cell or cell-matrix interaction [7, 8]. In contrast, the predicted product of *PKD2*, polycystin 2, shares some homology with the $\alpha_{1E\text{-}1}$ subunit of a voltage-activated calcium channel and is suggested to function as a channel by complexing with another protein, such as with polycystin 1 or with itself [9]. The mutation we described here is predicted to produce a truncated polycystin 2 lacking the EF-hand domain, resulting in a protein that would not bind calcium (Fig. 4). Moreover, recent studies have shown that polycystin 2 indeed heterodimerized with polycystin 1 and homodimerized with itself through two distinct C-terminal cytoplasmic domains [28, 29]. These interactions suggest that both polycystin 1 and 2 may function through a common signaling pathway that is essential for normal tubulogenesis. The same mutation is also predicted to produce a truncated protein lacking both the cytoplasmic domains required for polycystin 2 to interact with polycystin 1 and with itself [9, 28, 29]. Disruption of these interactions resulting from this mutation can conceivably be the basis for the cystic disease. Finally, frameshift mutations such as the one we described here can affect the stability of mutant mRNA and reduce the steady-state levels of PKD2 mRNA and protein [26].

Of the 25 different mutations reported in the unique region and part of the duplicated region of *PKD1*, most of them are nonsense and frameshift mutations [10–16]. In comparison, three nonsense and two frameshift (including that in the current report) mutations have been described for *PKD2* to date [9, 17]. In this limited data set, no clear mutation hot spot is apparent. Most of the *PKD1* and *PKD2* mutations reported would be predicted to result in premature translational termination and the production of a truncated protein [9–17]. It remains speculative whether these mutations will result in a loss of function or dominant negative effect [2, 16]. However, two recent reports have documented loss of heterozygosity at the *PKD1* locus in cystic epithelia and suggest that cystogenesis in ADPKD results from the inactivation of both alleles of *PKD1* from germline and somatic mutations [30, 31]. These data provide strong evidence favoring that most mutations in *PKD1* and perhaps *PKD2* may be inactivating. Structure-function studies of mutations in *PKD1* and *PKD2* should lead to a better understanding of the normal functions of these genes, as well as their role in the pathogenesis of ADPKD.

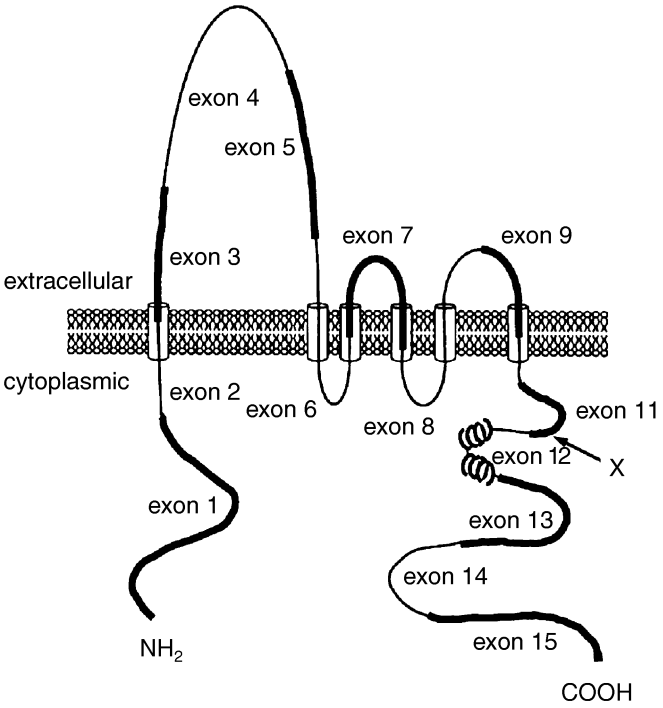

Fig. 4. A model of PKD2 gene product, polycystin 2, with correlation to exon structure [9, 24]. The six transmembrane spans predicted by the hydrophobicity plot are shown as cylinders within the cell membrane. The coil structures located in exon 12 denote the calcium-binding EF hand domain. The coiled-coil domain that mediates the interaction between polycystin 1 and 2 is located in exons 12 and 13 [28]. The position of a second coiled-coil domain for PKD2 self-dimerization has not been mapped precisely, but is located within exons 13-15 [29]. The mutation described in this report is predicted to result in a frameshift with premature translation termination of polycystin 2. The location of the stop codon resulting from this mutation is indicated by the symbol, X. The truncated protein is predicted to lack the EF-hand and the coiled-coil domains.

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REFERENCES

1. Fick G, Gabow P: Hereditary and acquired cystic disease of the kidney. *Kidney Int* 46:951–964, 1994
2. Harris PC, Ward CJ, Peral B, Hughes J: Polycystic kidney disease 1: Identification and analysis of the primary defect. *J Am Soc Nephrol* 6:1125–1133, 1995
3. Parfrey P, Bear J, Morgan J, Cramer B, McManamon P, Gault H, Churchill D, Singh M, Hewitt R, Somlo S, Reeders S: The diagnosis and prognosis of autosomal dominant polycystic kidney disease. *N Engl J Med* 323:1085–1090, 1990
4. Ravind D, Walker R, Gibson R, Forrest S, Richards R, Friend K, Sheffield L, Kincaid-Smith P, Danks D: Phenotype and genotype heterogeneity in autosomal dominant polycystic kidney disease. *Lancet* 340:1330–1333, 1992
5. Daoust MC, Reynolds DM, Bichet DG, Somlo S: Evidence for a third genetic locus for autosomal dominant polycystic kidney disease. *Genomics* 25:733–736, 1995
6. de Almeida S, de Almeida E, Peters D, Pinto J, Tavora I, Lavinha J, Breuning M, Prata M: Autosomal dominant polycystic kidney disease: Evidence for the existence of a third locus in a Portuguese family. *Hum Genet* 96:83–88, 1995
7. Hughes J, Ward C, Peral B, Aspinwall R, Clark K, San Millan J, Gamble V, Harris PC: The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. *Nature Genet* 10:151–160, 1995
8. International Polycystic Kidney Disease Consortium: Polycystic kidney disease: The complete structure of the PKD1 gene and its protein. *Cell* 81:289–298, 1995
9. Mochizuki T, Wu G, Hayashi T, Xenophontos S, Veldhuisen B, Saris JJ, Reynolds D, Cai Y, Gabow P, Pierides A, Kimberling W, Breuning M, Constantinou Deltas C, Peters D, Somlo S: PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272:1339–1342, 1996
10. Peral B, Gamble V, San Millan JL, Strong C, Sloane-Stanley J, Moreno F, Harris PC: Splicing mutations of the polycystic kidney disease 1 (PKD1) gene induced by intronic deletion. *Hum Mol Genet* 5:539–542, 1995
11. Turco AE, Rossetti S, Bresin E, Corra S, Gammaro L, Maschio G, Pignatti P: A novel nonsense mutation in the PKD1 gene (C3817T) is associated with autosomal dominant polycystic kidney disease (ADPKD) in a large three-generation Italian family. *Hum Mol Genet* 4:1331–1335, 1995
12. Peral B, Ong ACM, San Millan JL, Gamble V, Rees L, Harris PC: A stable nonsense mutation associated with a case of infantile onset polycystic kidney disease 1 (PKD1). *Hum Mol Genet* 5:539–542, 1996
13. Peral B, San Millan JL, Ong ACM, Gamble V, Ward CJ, Strong C, Harris PC: Screening the 3′ region of the polycystic kidney disease 1 (PKD1) gene reveals six novel mutations. *Am J Hum Genet* 58:86–96, 1996
14. Neophytou P, Constantinides R, Lazarou A, Pierides A, Constantinou Deltas C: Detection of a novel nonsense mutation and an intragenic polymorphism in the PKD1 gene of an autosomal dominant polycystic kidney disease Cypriot family. *Hum Genet* 98:437–442, 1996
15. Rossetti S, Bresin E, Restagno G, Carbonara A, Corra S, De Prisco O, Pignatti PF, Turco AE: Autosomal dominant polycystic kidney disease (ADPKD) in an Italian family carrying a novel nonsense mutation and two missense changes in exons 44 and 45 of the PKD1 gene. *Am J Med Genet* 65:155–159, 1996
16. Peral B, Gamble V, Strong C, Ong ACM, Sloan-Stanley J, Zerres K, Winearls C, Harris PC: Identification of mutations in the duplicated region of the polycystic kidney disease 1 (PKD1) gene by a novel approach. *Am J Hum Genet* 60:1399–1410, 1997
17. Xenophontos S, Constantinides R, Hayashi T, Mochizuki T, Somlo S, Pierides A, Deltas CC: A translation frameshift mutation induced by a cytosine insertion in the polycystic kidney disease 2 gene (PKD2). *Hum Mol Genet* 6:949–952, 1997
18. Miller S, Dykes D, Polesky H: Simple salting-out procedure for extracting DNA from human nucleated cells. (abstract) *Nucl Acid Res* 16:1215, 1987
19. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
20. Snarey A, Thomas S, Schneider M, Pound S, Barton N, Wright A, Somlo S, Germino G, Harris P, Reeders S, Frischauf A: Linkage disequilibrium in the region of the autosomal dominant polycystic kidney disease gene (PKD1). *Am J Hum Genet* 55:365–371, 1994
21. San Millan J, Viribay M, Peral B, Martinez I, Weissenbach J, Moren F: Refining the localization of the PKD2 locus on chromosome 4q by linkage analysis in Spanish families with autosomal dominant polycystic kidney disease. *Am J Hum Genet* 56:248–253, 1995
22. Terwilliger J, Ott J: *Handbook of Human Genetic Linkage*. Baltimore, Johns Hopkins University Press, 1994

23. Dobin A, Kimberling W, Pettinger W, Bailey-Wilson J, Shugart Y, Gabow P: Segregation analysis of autosomal dominant polycystic kidney disease. *Genet Epidemiol* 10:189–200, 1993
24. Hayashi T, Mochizuki T, Reynolds D, Wu Y, Cai Y, Somlo S: Characterization of the exon structure of the polycystic kidney disease 2 gene (PKD2). *Genomics* 44:131–136, 1997
25. Farber R, Petes T, Dominska M, Hudgens S, Liskay M: Instability of simple sequence repeats in a mammalian cell line. *Hum Mol Genet* 3:253–256, 1994
26. Strachan T, Read A: Mutation and instability of human DNA, in *Human Molecular Genetics*, Oxford, BIOS Scientific Publishers, Ltd., 1996, pp 252–255
27. Mahtani M, Willard H: A polymorphic X-linked tetranucleotide repeat locus displaying a high rate of new mutations: Implication for mechanisms of mutation at short tandem repeat locus. *Hum Mol Genet* 2:431–437, 1993
28. Qian F, Germino J, Cai Y, Zhang XB, Somlo S, Germino G: PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nature Genet* 16:179–183, 1997
29. Tsiokas L, Kim E, Arnould T, Sukhatme V, Walz G: Homo- and heterodimeric interactions between the gene products of PKD1 and PKD2. *Proc Natl Acad Sci USA* 94:6965–6970, 1997
30. Qian F, Watnick T, Onuchic L, Germino G: The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type 1. *Cell* 87:979–987, 1996
31. Brasier J, Henske E: Loss of the polycystic kidney disease (PKD1) region of chromosome 16p13.3 in renal cysts supports a loss-of-function model for cyst pathogenesis. *J Clin Invest* 99:194–199, 1997